Composition and plaque patterns of coronary culprit lesions and clinical characteristics of patients with chronic kidney disease

Keiji Kono[1], Hideki Fujii[1], Kentaro Nakai[1], Shunsuke Goto[1], Junya Shite[2], Ken-ichi Hirata[2], Masafumi Fukagawa[1,3] and Shinichi Nishi[1]

[1]*Division of Nephrology and Kidney Center, Kobe University Graduate School of Medicine, Kobe, Japan;* [2]*Division of Cardiovascular Medicine, Kobe University Graduate School of Medicine, Kobe, Japan and* [3]*Division of Nephrology and Metabolism, Tokai University School of Medicine, Isehara, Japan*

Coronary artery disease is a serious complication of chronic kidney disease (CKD); however, there is little information about coronary plaque morphology in these patients. Here we identified the characteristics of coronary culprit plaques and their clinical manifestations in 78 patients with CKD divided into four groups based on their estimated glomerular filtration rate. Patients were examined by Virtual Histology-Intravascular Ultrasound, a tomographic imaging method that can visualize atherosclerotic plaques *in vivo* using radiofrequency analysis of ultrasound backscatter signals. These ultrasound analyses showed an increase in the relative volumes of both dense calcium and necrotic core with decreasing renal function. The necrotic core/dense calcium ratio was significantly higher in patients with acute myocardial infarction compared to those with stable angina pectoris. Furthermore, the necrotic core/dense calcium ratio decreased in advanced CKD. Thus, the plaque composition of coronary culprit lesions changed from necrotic core-rich to extensively calcium-rich plaques as renal function decreased, suggesting that such coronary culprit composition was associated with stability, particularly in advanced CKD.

Kidney International (2012) **82,** 344–351; doi:10.1038/ki.2012.118; published online 18 April 2012

KEYWORDS: chronic kidney disease; coronary artery disease; plaque morphology; Virtual Histology-Intravascular Ultrasound

Correspondence: *Hideki Fujii, Division of Nephrology and Kidney Center, Kobe University Graduate School of Medicine, 7–5–2, Kusunoki-cho, Chuo-ku, Kobe, Hyogo 650–0017, Japan. E-mail: fhideki@med.kobe-u.ac.jp*

This study was partly presented at the annual meeting of the European Dialysis, Transplantation and Renal Association in 2011.

Received 14 November 2011; revised 19 January 2012; accepted 7 February 2012; published online 18 April 2012

Cardiovascular disease (CVD) is the leading cause of mortality in patients with chronic kidney disease (CKD).[1] It has been reported that these patients have more severe atherosclerotic lesions in coronary plaques as renal function decreases.[2,3] However, only a few studies have assessed coronary plaque composition in autopsy subjects. Although there are several imaging devices for the detection of coronary plaques, such as electron beam computed tomography, multidetector computed tomography, and magnetic resonance imaging, these devices provide only limited data about plaque morphology.

Virtual Histology-Intravascular Ultrasound (VH-IVUS) is a new tomographic imaging device that can visualize atherosclerotic plaques *in vivo* using radiofrequency analysis of ultrasound backscatter signals.[4] VH-IVUS can provide detailed information regarding the four major plaque components, and accuracy has been confirmed by comparison with histopathological findings (87.1% for fibrous, 87.1% for fibro fatty, 88.3% for necrotic core, and 96.5% for dense calcium).[5] This device is prevalently used not only in the decision-making process for coronary intervention in clinical settings but also in the assessment of coronary plaque morphology in clinical trials. There are accumulated evidences about the characteristics of coronary plaque composition and plaque stability using this imaging modality.[5–10] Our previous study demonstrated that hemodialysis (HD) patients had greater volumes of dense calcium and necrotic core on VH-IVUS analysis compared with control patients.[11] However, there are no studies providing detailed analysis of coronary culprit plaques in living patients with various degrees of renal function.

The objective of this study was to identify coronary culprit plaque characteristics using VH-IVUS analysis in patients with CKD, including HD patients.

RESULTS

Patient characteristics

Table 1 compares patient characteristics among the four groups. Age, body mass index, smoking, and the presence of diabetes and hyperlipidemia were comparable among the

Table 1 | Patient characteristics

Variables	eGFR (ml/min per 1.73 m^2) ⩾60 (*n*=31)	30–60 (*n*=24)	<30 (*n*=11)	HD (*n*=12)	*P*-value
Estimated GFR (ml/min per 1.73 m^2)	80.7 ± 15.4	47.1 ± 7.8[b]	20.9 ± 8.7[b,d]	—	<0.001
Age (years)	66 ± 11	70 ± 10	67 ± 13	65 ± 8	0.507
Sex (male; *n*, %)	15 (48)	21 (88)[b]	8 (73)	3 (25)	<0.005
Body mass index (kg/m^2)	23.7 ± 4.8	23.7 ± 2.9	26.5 ± 5.1	23.8 ± 3.8	0.242
Comorbidities (n, %)					
Smoking	10 (32)	7 (29)	2 (18)	4 (33)	0.831
Diabetes	15 (48)	9 (38)	7 (64)	9 (75)	0.151
Hypertension	17 (55)	18 (75)	10 (91)[a]	12 (100)[b]	<0.01
Hyperlipidemia	21 (68)	11 (46)	7 (64)	5 (42)	0.261
Diagnosis (n, %)					
Myocardial infarction	13 (42)	10 (42)	3 (28)	1 (8)	<0.01
Unstable angina pectoris	12 (39)	5 (21)	4 (36)	10 (84)[a,c]	
Stable angina pectoris	6 (19)	9 (37)	4 (36)	1 (8)	
Laboratory data					
Hemoglobin (g/dl)	12.7 ± 2.3	12.7 ± 1.8	11.4 ± 2.6	10.0 ± 1.9[b,d]	<0.005
Serum albumin (mg/dl)	3.7 ± 0.5	3.5 ± 0.5	3.2 ± 0.4[a]	3.4 ± 0.5	<0.01
LDL cholesterol (mg/dl)	110.1 ± 34.2	102.8 ± 27.1	115.4 ± 42.1	99.5 ± 39.0	0.662
HDL cholesterol (mg/dl)	43.2 ± 10.5	40.8 ± 11.6	44.3 ± 12.8	40.3 ± 14.1	0.765
Triglycerides (mg/dl)	152.1 ± 130.9	115.6 ± 52.2	133.8 ± 39.6	170.1 ± 119.3	0.431
Hemoglobin A1c (%)	6.3 ± 1.3	6.1 ± 1.3	6.3 ± 1.0	5.8 ± 1.1	0.584
C-reactive protein (mg/dl)	1.0 ± 2.0	1.3 ± 2.2	1.6 ± 1.6	2.5 ± 3.6	0.314
Serum calcium (mg/dl)	9.0 ± 0.5	8.6 ± 0.7[a]	8.6 ± 0.6	8.7 ± 0.5	<0.05
Serum phosphate (mg/dl)	3.3 ± 0.5	3.2 ± 0.6	4.0 ± 0.7[a,c]	4.8 ± 0.9[b,d]	<0.001
Calcium–phosphorus products (mg^2/dl^2)	30.5 ± 5.9	29.3 ± 6.6	38.0 ± 7.0[a,c]	44.5 ± 8.4[b,d]	<0.001
Intact-PTH (pg/ml)	—	—	—	271.3 ± 140.4	—
Medications (n, %)					
Phosphate binders	0 (0)	0 (0)	0 (0)	8 (67)[b,d,f]	<0.001
Calcium-containing	—	—	—	8	
Sevelamer hydrochloride	—	—	—	1	
Vitamin D	0 (0)	0 (0)	0 (0)	7 (58)[a,c,e]	<0.001
Renin–angiotensin system blocker	13 (42)	16 (67)	7 (64)	8 (67)	0.220
Statin	17 (55)	14 (58)	6 (55)	4 (33)	0.536

Abbreviations: eGFR, estimated glomerular filtration rate; HD, hemodialysis; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PTH, parathyroid hormone.
Vs. eGFR ⩾60: [a]$P<0.05$, [b]$P<0.01$. Vs. 30⩽ eGFR<60: [c]$P<0.05$, [d]$P<0.01$. Vs. eGFR <30: [e]$P<0.05$, [f]$P<0.01$.

four groups. Hypertension was more prevalent in the estimated glomerular filtration rate (eGFR) <30 ml/min per 1.73 m^2 and HD groups compared with the eGFR ⩾60 ml/min per 1.73 m^2 group. Serum hemoglobin levels were significantly lower in the HD group compared with the eGFR ⩾60 ml/min per 1.73 m^2 and 30⩽ eGFR <60 ml/min per 1.73 m^2 groups. Serum phosphate levels and calcium–phosphorus products were significantly higher in the eGFR <30 ml/min per 1.73 m^2 and HD groups compared with the eGFR ⩾60 ml/min per 1.73 m^2 and 30⩽ eGFR <60 ml/min per 1.73 m^2 groups.

Coronary angiography findings

Table 2 demonstrates the coronary angiography findings. The location of culprit lesions was similar among the four groups. Multivessel disease was more frequent, and single vessel disease was less frequent in the HD group compared with the other groups.

Table 2 | Coronary angiography findings

	eGFR (ml/min per 1.73 m^2) ⩾60 (*n*=31)	30–60 (*n*=24)	<30 (*n*=11)	HD (*n*=12)	*P*-value
Culprit lesion					
LAD (%)	13 (42)	10 (42)	4 (36)	5 (42)	0.812
LCX (%)	5 (16)	5 (20)	1 (9)	3 (25)	
RCA (%)	13 (42)	9 (38)	6 (55)	4 (33)	
Proximal (%)	25 (81)	19 (79)	9 (82)	9 (75)	0.556
Distal (%)	6 (19)	5 (21)	2 (18)	3 (25)	
Number of diseased vessels					
Single (%)	15 (48)	5 (21)	6 (55)	1 (8)[a]	<0.05
Double (%)	9 (29)	13 (54)	2 (18)	3 (25)	
Triple (%)	7 (23)	6 (25)	3 (27)	8 (67)	

Abbreviations: eGFR, estimated glomerular filtration rate; HD, hemodialysis; LAD, left anterior descending coronary artery; LCX, left circumflex coronary artery; RCA, right coronary artery.
Vs. eGFR ⩾60 ml/min per 1.73 m^2: [a]$P<0.05$.

Coronary culprit plaque morphology by VH-IVUS analysis

As demonstrated in Table 3, the volumes of both total plaque and vessel did not significantly differ among the four groups. With regard to coronary plaque composition, the relative volumes of dense calcium (%DC) and necrotic core (%NC) gradually increased with decreasing renal function (Figure 1).

Table 3 | Gray-scale intravascular ultrasound measurement

	eGFR (ml/min per 1.73 m^2)				
	⩾60 (*n*=31)	30–60 (*n*=24)	<30 (*n*=11)	HD (*n*=12)	*P*-value
Vessel volume (mm^3)	99.1 ± 27.6	95.8 ± 26.5	110.1 ± 48.0	110.3 ± 27.8	0.458
Lumen volume (mm^3)	32.4 ± 12.7	30.6 ± 10.8	35.7 ± 16.0	29.6 ± 6.7	0.629
Plaque volume (mm^3)	66.8 ± 21.0	65.3 ± 21.4	74.4 ± 36.5	80.6 ± 23.8	0.309
% Plaque volume (%)	66.8 ± 8.7	67.2 ± 9.5	66.3 ± 8.8	73.1 ± 4.7	0.247

Abbreviations: eGFR, estimated glomerular filtration rate; HD, hemodialysis.

%DC was significantly higher in the eGFR <30 ml/min per 1.73 m^2 and HD groups compared with the eGFR ⩾60 ml/min per 1.73 m^2 group ($P<0.05$). The NC/DC ratio, which was defined as %NC/%DC for assessment of plaque transformation, gradually decreased with the development of CKD (Figure 2).

Coronary plaque composition and risk factors

%NC was significantly and positively correlated with the presence of diabetes mellitus ($r=0.432$, $P<0.001$), hemoglobin A1c (HbA1c; $r=0.383$, $P<0.01$), serum phosphate levels ($r=0.234$, $P<0.05$), and calcium–phosphorus products ($r=0.253$, $P<0.05$), whereas %NC was significantly and negatively correlated with eGFR ($r=-0.280$, $P<0.05$). %DC was significantly and positively correlated with the presence of diabetes mellitus ($r=0.284$, $P<0.05$) and hypertension ($r=0.247$, $P<0.05$), serum phosphate levels ($r=0.464$, $P<0.0001$), and C-reactive protein ($r=0.254$, $P<0.05$), whereas %DC was significantly and negatively correlated with eGFR ($r=-0.454$, $P<0.001$). In addition, %DC tended to be correlated with serum albumin levels ($r=-0.218$, $P=0.06$) and HbA1c ($r=0.217$, $P=0.06$). In the multivariate analysis, the presence of diabetes mellitus and eGFR were significantly correlated with the extent of coronary plaque calcification (Table 4).

Coronary culprit plaque morphology and types of coronary artery disease

The NC/DC ratio was significantly higher in patients with acute myocardial infarction (AMI) than in those with stable angina pectoris (SAP; Figure 3). When patients were divided into two groups according to acute coronary syndrome (ACS) and SAP, the ratio was significantly higher in patients with ACS than in those with SAP (data not shown). Furthermore, the NC/DC ratio was higher in patients with AMI than in those with SAP even after stratification by renal function (Figure 4). The maximal arc of the calcium deposit, which indicates the size of clustered calcification in a coronary culprit plaque, gradually increased with decreasing renal function (Figure 5). Finally, total plaque volume was significantly higher in patients with AMI and unstable angina pectoris (UAP) than in those with SAP (AMI: 72.4 ± 22.2, UAP: 74.8 ± 27.8, SAP: 56.0 ± 15.9; $P<0.05$). The percentage of plaque volume (% plaque volume) was also significantly higher in patients with AMI and UAP than in those with SAP (AMI: 69.3 ± 8.8%, UAP: 69.9 ± 6.9%, SAP: 61.8 ± 8.7%; $P<0.05$).

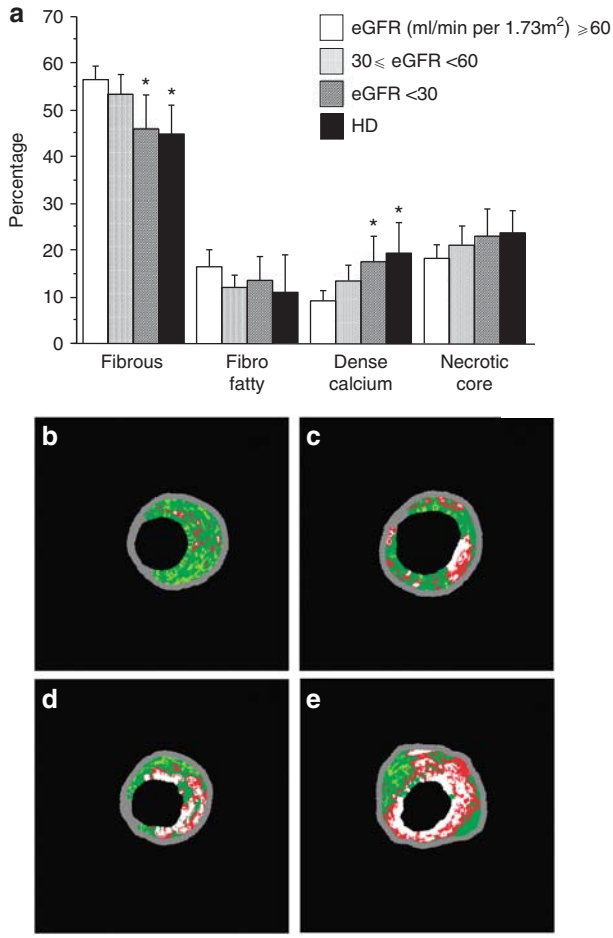

Figure 1 | Compositional pattern of coronary culprit lesions. (**a**) Coronary plaque morphology of the culprit lesion. HD, hemodialysis. Vs. eGFR (ml/min per 1.73 m^2) ⩾60, *$P<0.05$. Virtual Histology-Intravascular Ultrasound images of coronary culprit plaque. eGFR (ml/min per 1.73 m^2) (**b**) ⩾60, (**c**) 30–60, and (**d**) <30, and (**e**) hemodialysis group. Green, fibrous; yellow, fibro fatty; red, necrotic core; white, dense calcium. eGFR, estimated glomerular filtration rate.

DISCUSSION

The results of this study demonstrated several important findings, which are as follows: (1) the relative volumes of both DC and NC gradually increased and the NC/DC ratio gradually decreased in coronary culprit lesions with the development of CKD; (2) the NC/DC ratio was significantly higher in patients with AMI compared with those with SAP; and (3) calcified lesions of coronary plaque gradually developed in clusters as renal function decreased.

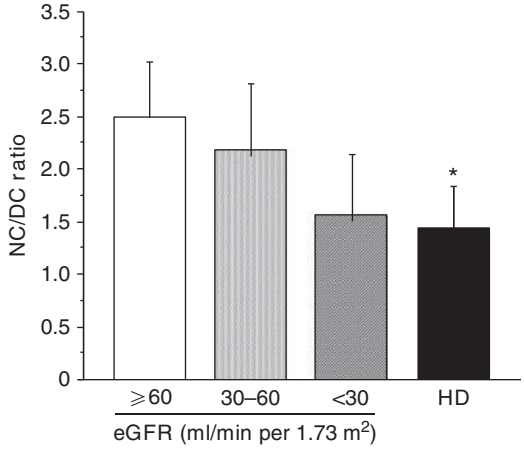

Figure 2 | Necrotic core (NC)/dense calcium (DC) ratio in the coronary culprit lesion. eGFR, estimated glomerular filtration rate; HD, hemodialysis. Vs. eGRF (ml/min per 1.73 m^2) ⩾60, *$P<0.05$.

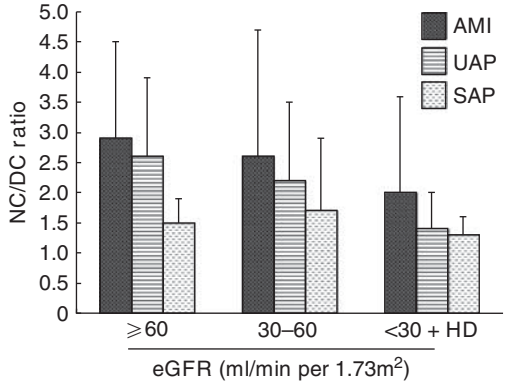

Figure 4 | Comparison of necrotic core (NC)/dense calcium (DC) ratio among different types of coronary artery disease in each group stratified by renal function. AMI, acute myocardial infarction; eGFR, estimated glomerular filtration rate; HD, hemodialysis; SAP, stable angina pectoris; UAP, unstable angina pectoris.

Table 4 | Multivariate analysis between the relative volume of dense calcium and clinical parameters

Variables	β	*P*-value
Age (years)	0.029	0.795
Diabetes (yes vs. no)	0.220	<0.05
Hypertension (yes vs. no)	0.088	0.427
eGFR (ml/min per 1.73 m^2)	−0.308	<0.05
Serum albumin (mg/dl)	0.108	0.372
C-reactive protein (mg/dl)	0.220	0.062
Calcium–phosphorus products (mg^2/dl^2)	0.144	0.307

Abbreviation: eGFR, estimated glomerular filtration rate.

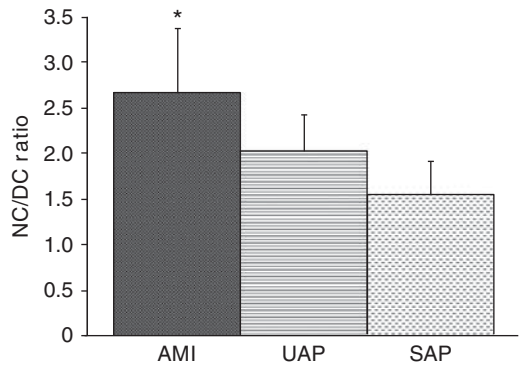

Figure 3 | Comparison of necrotic core (NC)/dense calcium (DC) ratio among different types of coronary artery disease (CAD) in all the study patients. AMI, acute myocardial infarction; SAP, stable angina pectoris; UAP, unstable angina pectoris. Vs. SAP, *$P<0.05$.

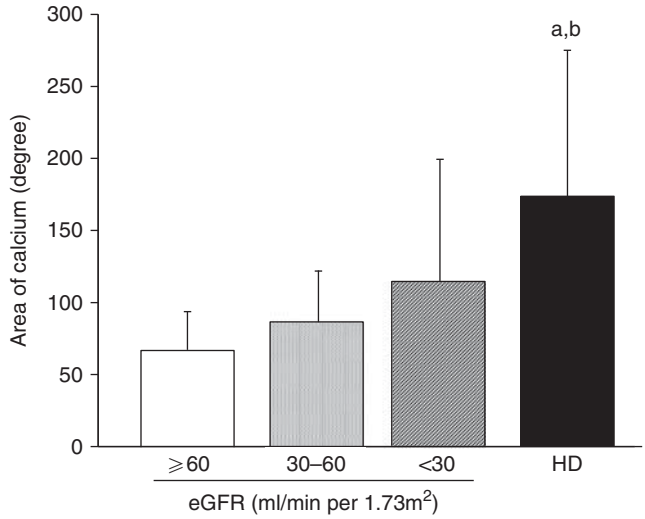

Figure 5 | Maximal arc of calcium deposit in the culprit lesion. HD, hemodialysis. Vs. eGFR (ml/min per 1.73 m^2) ⩾60: $^aP<0.05$ vs. 30⩽ eGFR <60: $^bP<0.05$. eGFR, estimated glomerular filtration rate.

There are many previous studies that have shown that CKD patients have severe atherosclerosis and that they are at high risk for CVD.[2,3,12–14] However, only a few previous studies have outlined the characteristics of coronary plaques in detail. Two recent reports from autopsy subjects demonstrated changes in coronary plaque morphology with decreasing renal function.[2,3] Nakamura *et al.*[2] demonstrated that coronary plaque calcification was more severe in patients with CKD stages 3, 4–5, and 5D compared with those without CKD. Nakano *et al.*[3] also reported that atherosclerosis was more advanced in patients with eGFR <45 ml/min per 1.73 m^2 according to the classification by the Committee on Vascular Lesions of the Council on Atherosclerosis, American heart association. However, these autopsy studies were considered to be limited particularly in terms of patient selection. On the other hand, several imaging devices, such as computed tomography and magnetic resonance imaging, have been prevalently used for the assessment of coronary artery disease (CAD) in living patients. However, detailed analysis of coronary plaque composition cannot be performed by computed tomography and magnetic resonance imaging, even though the resolution of these noninvasive imaging devices has improved. IVUS enables visualization of the atherosclerotic plaque with high accuracy, and

provides information about coronary plaque morphology.[5–11] Therefore, we used VH-IVUS to examine changes in coronary culprit plaque morphology and their association with clinical parameters in living CKD patients in this study.

In their study using VH-IVUS, Ogita *et al.*[15] reported that %NC and %DC in non-culprit coronary artery lesions increased with decreasing renal function in diabetic CKD patients. A recent study using Integrated Backscatter IVUS demonstrated that coronary culprit plaques in patients with eGFR <60 ml/min per 1.73 m^2 included higher percentage of lipid volume and lower percentage of fibrous volume compared with those with an eGFR $\geqslant 60$ ml/min per 1.73 m^2. However, average renal function in the eGFR <60 ml/min per 1.73 m^2 group was 49 ± 9 ml/min per 1.73 m^2 in the study. Therefore, it seems that the lower eGFR group mainly included CKD stage 3 patients.[16] Thus, there is relatively little data regarding coronary culprit plaque morphology in living patients with various degrees of renal function. In the present study, %NC and %DC in culprit lesions of the coronary artery gradually increased with decreasing renal function. Conversely, the NC/DC ratio decreased with decreasing renal function. In addition, %NC was significantly correlated with diabetes mellitus, eGFR, and serum phosphorus levels. %DC was significantly correlated with multiple risk factors, such as the presence of diabetes mellitus and hypertension, eGFR, mineral disorders, and inflammation. From these results, we speculated that NC and calcified lesions in atherosclerotic plaques are induced in patients with decreasing renal function not only by traditional risk factors, such as diabetes and hypertension, but also by nontraditional CKD-related risk factors, such as uremic toxins and mineral disorders. Furthermore, it is suggested that the decrease in the NC/DC ratio with reducing renal function reflects plaque transformation from NC-rich plaques to calcium-rich plaques.

Several studies have reported that vascular calcification is markedly severe in CKD patients and the incidence of CVD increases with decreasing renal function.[17,18] It is well known that the link between renal dysfunction and vascular calcification is very important in cardio-renal association and both are also crucial predictors of CVD.[19,20] Interestingly, US Renal Data System Data annual reports show that only 20% of deaths related to CVD in dialysis patients were caused by myocardial infarction.[21] Heart failure and arrhythmia rather than AMI were frequently observed in advanced CKD patients. Taking these facts into account, we postulated that vascular calcification might be associated with a type of CVD in CKD patients.

Pathological analysis of coronary plaques in the general population demonstrated that ACS mainly resulted from rupture of the vulnerable plaques, whereas calcified nodules were least common. Such vulnerable plaques consisted of a higher percentage of NC compared with stable plaques.[22] Previous studies using VH-IVUS analysis also demonstrated that a higher NC/DC ratio was associated with ACS.[7,8] Although direct evidence regarding the relationship between the NC/DC ratio and plaque stability may be absent, previous studies have reported that NC decreases plaque stability, whereas calcification increases its stability.[7,8,22–24] In addition, previous studies demonstrated an association between the pattern of plaque calcification and plaque stability. Beckman *et al.*[9] reported that the maximal arc of the calcium deposits in coronary culprit plaques was significantly lower in patients with AMI compared with those with SAP. Using IVUS, Ehara *et al.*[10] also demonstrated that spotty calcification was most frequent in AMI patients, whereas extensive calcification was most frequent in SAP patients. Because the junction between calcified and non-calcified lesions in atherosclerotic plaques is thought to be weak against mechanical stress, the pattern of calcium deposition may be an important indicator for plaque stability.[25]

On the basis of the hypothesis that the NC/DC ratio and the pattern of calcification in coronary artery plaques could be indicators of plaque stability, we examined the association of coronary plaque morphology with a type of CAD in CKD patients. In this study, the NC/DC ratio was significantly higher in patients with AMI compared with those with SAP, and this tendency remained even after stratification by renal function. In addition, the maximal arc of the calcium deposit gradually increased with decreasing renal function. These results indicate that the transformation from NC-rich plaques into extensively calcium-rich plaques in coronary culprit lesions may lead to plaque stability along with declining renal function.

Previous studies have demonstrated that CKD patients had coronary microvascular dysfunction despite the absence of obstructive CAD.[26,27] Furthermore, recent studies demonstrated an association between impaired coronary microvascular circulation and coronary artery calcification score in HD patients.[28,29] Coronary microvascular circulation regulates the balance between coronary blood flow supply and myocardial demand, and its dysfunction could be assessed by measuring coronary flow reserve in response to adenosine-triphosphate stress. Our previous study demonstrated that coronary flow reserve is positively and significantly associated with renal function.[27] In addition, reduced coronary flow reserve is associated with the development of myocardial ischemia and cardiovascular mortality.[26,30] Although this was not the focus of our study, coronary microvascular dysfunction, which is supposed to be associated with both decreasing renal function and the increasing extent of coronary artery calcification, may have an important role in the development of cardiac abnormalities in CKD patients. In fact, heart failure is the most frequent CVD in HD patients.[31] Therefore, we attributed the increased prevalence of CVD death, particularly in advanced CKD patients, to impairment of coronary microvascular circulation related to vascular calcification.

Previous studies suggested that increased plaque burden and lipid plaque volume were associated with the occurrence of ACS.[32,33] Results of this study showed that increased plaque volume was associated with ACS; however, the relationship between plaque volume and renal function

could not be identified. Two autopsy studies demonstrated that coronary plaque volume was comparable between CKD patients and control patients, and there was no autopsy study that investigated plaque volume in patients with varying renal function.[12,13] Furthermore, a previous study using VH-IVUS analysis demonstrated that plaque volume was comparable among patients with varying renal function.[15] Rigatto *et al.*[34] suggested that plaque growth may be driven not only by impaired renal function but also by other factors, such as hypertension and diabetes. Taking these facts into account, the change in plaque volume remains controversial in CKD patients, and longitudinal studies are needed to resolve this issue. With regard to the association between hyperlipidemia and CVD, randomized controlled trials of statin therapies in HD patients, such as 4-D and AURORA trials, have failed to show their effects on CVD outcomes.[35,36] The present study also could not identify a significant relationship between lipid plaque volume and statin therapy (data not shown). These results could be partly explained by the lipid metabolism disorder specific to CKD patients. In fact, the present study showed that serum low-density lipoprotein levels were lowest in the HD group.

This study has some limitations. First, in the setting of the study design inherence, this study could not establish a cause–effect relationship. A longitudinal study is needed in the future to resolve this issue. Second, the number of study participants was relatively small, especially when patients were divided into four groups according to the eGFR. Certainly, if we were able to evaluate more patients, the results would be more conclusive. Third, VH-IVUS was performed for the decision-making process of coronary intervention, and this study did not include the information about coronary plaque other than the culprit lesion. Furthermore, in general, it is difficult to analyze IVUS in patients with increased volume of calcification. Therefore, we excluded two patients with insufficient images from VH-IVUS analysis. However, considering that there were only a few studies to assess the detailed coronary plaque morphology, especially in living CKD patients, we believe that our study is potentially valuable in understanding the pathophysiology of CVD in CKD patients.

In conclusion, our findings suggested that the compositional plaque pattern in coronary culprit lesion was transformed from necrotic-rich plaques into calcium-rich plaques with the development of CKD, and that its composition was associated with its stability. In addition, VH-IVUS was thought to be a useful device not only for determining optimal strategies for coronary intervention but also for understanding the pathophysiology of CVD in CKD.

MATERIALS AND METHODS

Study population and design

A total of 950 patients had undergone coronary angiography and VH-IVUS between May 2005 and December 2009 at our institution. Of these patients, 813 were excluded because they did not have sufficient records of clinical laboratory examinations and/or VH-IVUS for their evaluation. Forty-nine patients who had lesions after coronary bypass grafting or percutaneous coronary intervention were also excluded. Furthermore, patients with overt infection (three patients), malignancy (five patients), and inflammatory disease (two patients) were also excluded (Figure 6). The remaining 78 patients were included in this study. Of these patients, 23 were also included in our previous study.[11] The 78 study patients were classified into four groups based on their eGFR (eGFR $\geqslant$60 (ml/min per 1.73 m^2), $n=31$; 30$\leqslant$ eGFR $<$60, $n=24$; eGFR $<$30, $n=11$; HD, $n=12$).

For the diagnosis of CAD, AMI was defined as prolonged chest pain with typical electrocardiographic changes and enzyme elevation (creatine kinase (CK) or CK-MB $>$2 times the upper limit of the normal range) within 24 h after the onset of symptoms.[37] UAP was defined as new-onset angina (within 2 months) or accelerated angina (more severe, frequent, or longer in duration) or angina at rest, whereas SAP was defined as typical chest pain on exertion.[38]

Diabetes mellitus was defined as a fasting blood glucose level $\geqslant$126 mg/dl or the use of antidiabetic medications (insulin or oral hypoglycemics). Hypertension was defined as systolic blood pressure $\geqslant$140 mm Hg, diastolic blood pressure $\geqslant$90 mm Hg, or the use of antihypertensive drugs. Hyperlipidemia was defined as a present or past low-density lipoprotein cholesterol level $\geqslant$140 mg/dl or the use of statin. Experimental protocols were approved by the appropriate institutional review committee, and informed consent was obtained from all subjects.

VH-IVUS imaging and analysis

As described in our previous study,[11] we analyzed the culprit lesion, which was defined as one of the most stenotic lesions responsible for clinical symptoms and results of examinations, such as echocardiographic abnormalities and/or positive findings of stressed electrocardiogram and/or myocardial scintigraphy in this study. All VH-IVUS examinations were performed before PCI and after intracoronary administration of 300 μg nitroglycerin. VH-IVUS was not performed when patients had lesions that were difficult to cross the IVUS catheter. Furthermore, if the lesion showed angiographic signs of thrombus, thrombus aspiration was performed to avoid incorrect identification of a thrombus as a coronary plaque. The VH-IVUS catheter was automatically pulled back at a rate of 0.5 mm/s throughout the culprit lesion. During pullback, all IVUS images were recorded. We reviewed these images on a display, and the vessel and lumen areas of each selected culprit lesion were traced for every recorded frame. From these profiles, plaque composition was analyzed using a custom-built software (Volcano Therapeutics, Rancho Cordova, CA). Results were expressed as the relative and absolute volumes of four major plaque components: fibrous, fibro fatty, DC, and NC.

Using the results of the VH-IVUS analysis, the percentage of plaque volume (% plaque volume) was calculated as follows: (total plaque volume/vessel volume) $\times$ 100 (%). Furthermore, we calculated the NC/DC ratio as %NC/%DC for assessment of plaque transformation, which was based on the method described by Missel *et al.*[7] In each patient, the angle of the largest calcium deposit, which was chosen in each assessed plaque area, was measured with a protractor centered on the lumen, and it was defined as 'maximal arc of calcium deposit' by reference to the method described in the previous report.[9] For assessment of reproducibility, inter-rater reliability was measured by calculating the intraclass correlation coefficient. The inter-observer intraclass correlation coefficient was 0.98 for three observers.

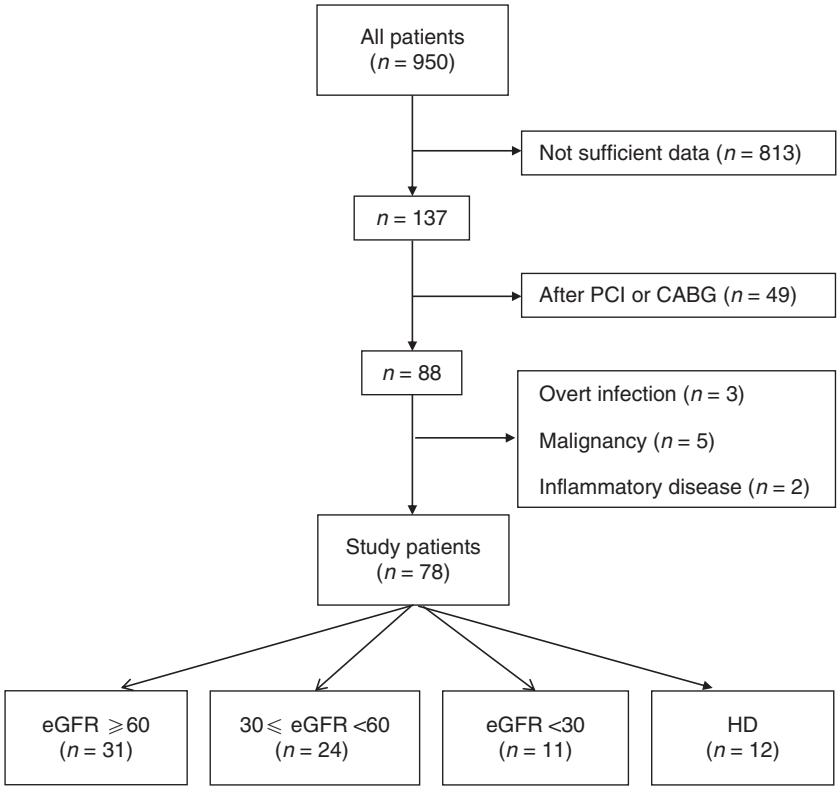

Figure 6 | Study population flowchart. CABG, coronary artery bypass graft; eGFR, estimated glomerular filtration rate; HD, hemodialysis; PCI, percutaneous coronary intervention.

Measurement of biomarker by clinical laboratory tests

Venous blood was collected from each patient following a 20-min period of supine rest in the morning after overnight fasting. In the HD group, blood was collected at the beginning of the first dialysis session of each week according to the Japanese society of dialysis therapy guideline. Laboratory tests were conducted according to standardized clinical laboratory methods. The blood chemistry data were calculated as the average of three measurements. To assess renal function, we applied the following modified version of the Modification of Diet in Renal Disease equation for evaluating Japanese CKD patients:[39] eGFR (ml/min per 1.73 m^2) = 0.741 × 175 × (serum creatinine, mg/dl)$^{-1.154}$ × (age, years)$^{-0.203}$ × (0.742 for women).

Statistical analysis

All statistical analyses were performed using StatView 5.0. for windows (SAS Institute, Cary, NC). All values are expressed as mean ± s.d. A comparison of patient characteristics and IVUS analyses between the groups was performed using the χ^2-test (for categorical variables) and one-way analysis of variance (for continuous variables), followed by the Games-Howell *post hoc* analysis. Univariate and multivariate regression analyses were performed to assess the contributors to the development of each plaque composition. Values were described as mean ± s.d., and $P<0.05$ was considered to be statistically significant.

DISCLOSURE

All the authors declared no competing interests.

REFERENCES

1. Deo R, Fyr CL, Fried LF *et al.* Health ABC study. Kidney dysfunction and fatal cardiovascular disease an association independent of atherosclerotic events: results from the Health, Aging, and Body Composition (Health ABC) study. *Am Heart J* 2007; **155**: 62–68.
2. Nakamura S, Ishibashi-Ueda H, Niizuma S *et al.* Coronary calcification in patients with chronic kidney disease and coronary artery disease. *Clin J Am Soc Nephrol* 2009; **4**: 1892–1900.
3. Nakano T, Ninomiya T, Sumiyoshi S *et al.* Association of kidney function with coronary atherosclerosis and calcification in autopsy samples from Japanese elders: the Hisayama study. *Am J Kidney Dis* 2010; **55**: 21–30.
4. Murray SW, Stables RH, Palmer ND. Virtual histology imaging in acute coronary syndromes: useful or just a research tool? *J Invasive Cardiol* 2010; **22**: 84–91.
5. Nasu K, Tsuchikane E, Katoh O *et al.* Accuracy of *in vivo* coronary plaque morphology assessment: a validation study of *in vivo* virtual histology compared with *in vitro* histopathology. *J Am Coll Cardiol* 2006; **47**: 2405–2412.
6. Nasu K, Tsuchikane E, Katoh O *et al.* Plaque characterization by virtual histology intravascular ultrasound analysis in patients with type 2 diabetes. *Heart* 2008; **94**: 429–433.
7. Missel E, Mintz GS, Carlier SG *et al. In vivo* virtual histology intravascular ultrasound correlates of risk factors for sudden coronary death in men: results from the prospective, multi-centre virtual histology intravascular ultrasound registry. *Eur Heart J* 2008; **29**: 2141–2147.
8. Missel E, Mintz GS, Carlier SG *et al.* Necrotic core and its ratio to dense calcium are predictors of high-risk non-ST elevation acute coronary syndrome. *Am J Cardiol* 2007; **101**: 573–578.
9. Beckman JA, Ganz J, Creager MA *et al.* Relationship of clinical presentation and calcification of culprit coronary artery sternoses. *Aterioscler Thromb Vasc Biol* 2001; **21**: 1618–1622.
10. Ehara S, Kobayashi Y, Yoshiyama M *et al.* Spotty calcification typifies the culprit plaque in patients with acute myocardial infarction: an intravascular ultrasound study. *Circulation* 2004; **110**: 3424–3429.
11. Kono K, Fujii H, Miyoshi N *et al.* Coronary plaque morphology using virtual histology-intravascular ultrasound analysis in hemodialysis patients. *Ther Apher Dial* 2011; **15**: 44–50.
12. Gross ML, Meyer HP, Ziebart H *et al.* Calcification of coronary intima and media: immunohistochemistry, backscatter imaging, and X-ray analysis in renal and nonrenal patients. *Clin J Am Soc Nephrol* 2007; **2**: 121–134.

13. Schwarz U, Buzello M, Ritz E *et al.* Morphology of coronary atherosclerotic lesions in patients with end-stage renal failure. *Nephrol Dial Transplant* 2000; **15**: 218–223.
14. McCullough PA, Agrawal V, Danielewicz E *et al.* Accelerated atherosclerotic calcification and Monckeberg's sclerosis: a continuum of advanced vascular pathology in chronic kidney disease. *Clin J Am Soc Nephrol* 2008; **3**: 1585–1598.
15. Ogita M, Funayama H, Nakamura T *et al.* Plaque characterization of non-culprit lesions by virtual histology intravascular ultrasound. *J Cardiol* 2009; **54**: 59–65.
16. Miyagi M, Ishii H, Murakami R *et al.* Impact of renal function on coronary plaque composition. *Nephrol Dial Transplant* 2010; **25**: 175–181.
17. Go AS, Chertow GM, Fan D *et al.* Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med* 2004; **351**: 1296–1305.
18. Ninomiya T, Kiyohara Y, Kubo M *et al.* Chronic kidney disease and cardiovascular disease in a general Japanese population: the Hisayama Study. *Kidney Int* 2005; **68**: 228–236.
19. Detrano R, Guerci AD, Carr JJ *et al.* Coronary calcium as a predictor of coronary events in four racial or ethnic groups. *N Engl J Med* 2008; **358**: 1336–1345.
20. Pletcher MJ, Tice JA, Pignone M *et al.* Using the coronary artery calcium score to predict coronary heart disease events: a systematic review and meta-analysis. *Arch Intern Med* 2004; **28**: 1285–1292.
21. United States Renal Data System. USRDS 1999 annual data report. National Institutes of Health, National Institutes of Diabetes and Digestive and Kidney Disease, 1999.
22. Virmani R, Bruke AP, Farb A *et al.* Pathology of the vulnerable plaque. *J Am Coll Cardiol* 2006; **47**: C13–C18.
23. Finn AV, Nakano M, Naruta J *et al.* Concept of vulnerable plaque/unstable plaque. *Arterioscler Thromb Vasc Biol* 2010; **30**: 1282–1292.
24. Fall K, Maehara A, Mintz GS. Intravascular imaging in patients with acute coronary syndromes and unstable coronary plaques. *Curr Cardiovasc Imaging Rep* 2011; **4**: 269–275.
25. Abedin M, Tintut Y, Demer LL. Vascular calcification: mechanisms and clinical ramifications. *Atherioscler Thromb Vasc Biol* 2004; **24**: 1161–1170.
26. Chade AR, Brosh D, Higano ST *et al.* Mild renal insufficiency is associated with reduced coronary flow in patients with non-obstructive coronary artery disease. *Kidney Int* 2006; **69**: 266–271.
27. Fujii H, Takiuchi S, Kawano Y *et al.* Putative role of asymmetric dimethylarginine in microvascular disease of kidney and heart in hypertensive. *Am J Hypertens* 2008; **21**: 650–656.
28. Caliskan Y, Demirturk M, Ozkok A *et al.* Coronary artery calcification and coronary flow velocity in haemodialysis patients. *Nephrol Dial Transplant* 2010; **25**: 2685–2690.
29. Caliskan Y, Olfaz H, Demirturk M *et al.* Coronary flow reserve dysfunction in hemodialysis and kidney transplant patients. *Clin Transplant* 2008; **22**: 785–793.
30. Opherk D, Mall G, Zebe H *et al.* Reduction of coronary reserve: a mechanism for angina pectoris in patients with arterial hypertension and normal coronary arteries. *Circulation* 1984; **69**: 1–7.
31. Harnett JD, Foley RN, Kent GM *et al.* Congestive heart failure in dialysis patients: prevalence, incidence, prognosis and risk factors. *Kidney Int* 1995; **47**: 884–890.
32. Nicholls SJ, Hsu A, Wolski K *et al.* Intravascular ultrasound-derived of coronary atherosclerotic plaque burden and clinical outcome. *J Am Coll Cardiol* 2010; **55**: 2399–2407.
33. Naghavi M, Libby P, Falk E *et al.* From vulnerable plaque to vulnerable patient: a call for new definitions and risk assessment strategies: Part I. *Circulation* 2003; **108**: 1664–1672.
34. Rigatto C, Levin A, House AA *et al.* Atheroma progression in chronic kidney disease. *Clin J Am Soc Nephrol* 2009; **4**: 291–298.
35. Wanner C, Krane V, Marz W *et al.* Atorvastatin in patients with type 2 diabetes mellitus undergoing hemodialysis. *N Engl J Med* 2005; **353**: 238–248.
36. Fellstrom BC, Jardine AG, Schmieder RE *et al.* Rosuvastatin and cardiovascular events in patients undergoing hemodialysis. *N Engl J Med* 2009; **360**: 1395–1407.
37. Alpert JS, Thygesen K, Antman E *et al.* Myocardial infarction redefined a consensus document of the Joint European Society of Cardiology/ American College of Cardiology Committee for the redefinition of myocardial infarction. *J Am Coll Cardiol* 2000; **36**: 959–969.
38. Braunwald E. Unstable angina. A classification. *Circulation* 1989; **80**: 410–414.
39. Matsuo S, Imai E, Horio M *et al.* Collaborators developing the Japanese equation for estimated GFR: Revised equations for estimated GFR from serum creatinine in Japan. *Am J Kidney Dis* 2009; **53**: 982–992.